Jpn. J. Cancer Res. (Gann), 79, 866–873 ; July, 1988

In vitro Augmentation of Natural Killer Cell Activity and Production of Interferon-α/β and -γ with Deoxyribonucleic Acid Fraction from *Mycobacterium bovis* BCG

Saburo YAMAMOTO,[*1, *3] Etsuro KURAMOTO,[*2] Shizuo SHIMADA[*2] and Tohru TOKUNAGA[*1]
[*1]*Department of Cellular Immunology, National Institute of Health, 2-10-35, Kamiosaki, Shinagawa-ku, Tokyo 141 and* [*2]*Institute of Biological Science, Mitsui Pharmaceuticals, Inc., Togo, Mobara-shi, Chiba-ken 297*

A nucleic acid-rich fraction extracted and purified from BCG (MY-1) augmented natural killer (NK) cell activity of mouse spleen cells *in vitro*, and produced factor(s) which showed anti-viral activity and rendered normal macrophages cytotoxic towards tumor cells. These cellular responses were induced by the MY-1 digested preliminarily with RNase, but not by the MY-1 digested with DNase, indicating that DNA contained in MY-1 was essential for the responses. The function of the factor to activate macrophages was destroyed by treatment with a small amount of anti-interferon (IFN)-γ antiserum or under acidic conditions (pH 2), but not by treatment with anti-IFN-α/β antiserum, while the anti-viral activity was destroyed almost completely by treatment with anti-IFN-α/β antiserum. It appears that DNA from BCG stimulated mouse spleen cells *in vitro*, resulting in augmentation of NK activity and production of IFN-α/β and -γ.

Key words: BCG — DNA — Interferon-α/β — Interferon-γ — Natural killer

In the previous papers,[1, 2] we reported that a fraction extracted and purified from *Mycobacterium bovis* BCG (MY-1), which was composed of 70.0% DNA, 28.0% RNA, 1.3% protein, 0.27% hexose and 0.1% lipid, exhibited strong antitumor activity against nine different syngeneic mouse tumors and one guinea pig tumor. The antitumor activity was ascribed to the DNA contained in MY-1, because DNase-digested MY-1, which was composed of 97.0% RNA, showed a much reduced activity, whereas RNase-digested MY-1, which contained 97.0% DNA, was more effective than the undigested MY-1.[1, 2] MY-1 showed no direct cytotoxicity *in vitro* against those tumors,[1] and the main effector cells responsible for the antitumor activity seemed to be the natural killer (NK)[*4] cells activated by MY-1 *in vivo*.[3]

Recently, we found that when normal mouse spleen cells were incubated with MY-1 *in vitro*, NK activity was remarkably augmented, and the spleen cells produced factors that were capable of inhibiting cytopathic effects of vesicular stomatitis virus (VSV) and also of activating normal macrophages to become cytotoxic towards tumor cells. In this paper, we describe the active entity in MY-1 responsible for these *in vitro* activities, and the properties of the factors produced by the cells.

MATERIALS AND METHODS

MY-1 and Its Nuclease-digested Fractions Methods of preparation of MY-1, and the RNase- or DNase-digested MY-1, were described previously.[1] The lyophilized preparations were dissolved in distilled water before use.

Antisera and Other Reagents Anti-asialo GM_1 antiserum was prepared as described previously.[3] Low-toxicity rabbit complement was purchased from Cedarlane Lab. Ltd., Hornby, Ontario, Canada. Anti-mouse IFN-γ antiserum (anti-IFN-γ) and monoclonal anti-mouse IFN-γ antibody were kindly provided by Dr. Y. Watabe and Prof. Y. Kawade (Institute for Virus Research, Kyoto University, Kyoto), and by Dr. R. D. Schreiber (Research Institute of Scripps Clinic, La Jolla, CA), respectively. Standard mouse IFN-α/β and rabbit anti-mouse IFN-α/β antiserum were purchased from Lee Biomolecular Research Lab., Inc. (San Diego, CA). Bacterial lipopolysaccharide (LPS;

[*3] To whom all correspondence should be addressed.

[*4] Abbreviations used: IFN, interferon; MAF, macrophage-activating factor; NK, natural killer; PBS, phosphate-buffered saline; polyI: polyC, polyinosinic-polycytidylic acid; VSV, vesicular stomatitis virus.

E. coli, Serotype 0111:B4; Difco Lab., Detroit, MI), polyinosinic-polycytidylic acid (polyI:polyC; Yamasa Shoyu Co., Ltd., Choshi Chiba) and Sephadex G-10 (Pharmacia Fine Chemicals, Uppsala, Sweden) were purchased.

Preparation of Spleen Cells Suspensions of spleen cells from 3–5 BALB/c mice were prepared in RPMI 1640 medium supplemented with 10% fetal bovine serum (complete medium) by passage through 200-mesh stainless steel sieves. The cell suspensions were placed in tubes with distilled water for 5–10 sec to lyse red blood cells. The spleen cell suspensions were then washed with Hanks' balanced salt solution and resuspended in complete medium.

Pretreatment of Spleen Cells with Antisera Adherent cells were removed by passage of spleen cells ($2–5\times10^8$ cells) through a column of 10 g (dry weight) of Sephadex G-10 in a 60-ml plastic syringe. The nonadherent cells were eluted with 40 ml of warm complete medium. Residual macrophages in this fraction were less than 0.6% when measured by latex bead ingestion assay.

Preparation of Spleen Adherent Cells Aliquots (1 ml each) of spleen cell suspension at a concentration of 5×10^6/ml were poured into plastic dishes and incubated at 37° for 2 hr. Nonadherent cells were removed by intensive washing of the monolayers with warm complete medium. The washing was repeated twice more, and the dishes were rinsed with dication-free phosphate-buffered saline (PBS). The adherent cells were incubated with PBS containing 0.5m*M* EDTA at 37° for 15 min, and the cells were harvested. The adherent cells obtained comprised more than 70% macrophages when measured by assaying phagocytosis of latex beads.

***In vitro* Activation of Spleen Cells with MY-1** Spleen cells (1×10^7/ml) were incubated with either MY-1, DNase digest of MY-1, or RNase digest of MY-1 in 24-well tissue culture plates (Costar, Cambridge, MA) at 37° for various time periods in a 5% CO_2 incubator. After the incubation, the culture supernatants were collected and tested for macrophage-activating and anti-viral activities. The precipitated cells were washed, resuspended in complete medium and assayed for NK activity.

Assay for NK Activity RL♂1 mouse lymphoma cells maintained as suspension cultures in complete medium were used as target cells. Effector spleen cells were incubated with ^{51}Cr-labeled target cells (1×10^4) in flat-bottomed 96-well plastic plates for 4 hr at 37°. After the incubation, the plates were centrifuged at 800*g* for 10 min, and the radioactivity in 0.1 ml of the supernatants was measured with a gamma-scintillation counter. All combinations were performed in triplicates. The percentage of specific lysis was calculated by applying the following formula:

$$\% \text{ Lysis} = \frac{\text{test cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100,$$

where test cpm was the mean cpm released in the presence of effector cells, spontaneous cpm was the mean cpm released by target cells alone, and total cpm was the total amount of ^{51}Cr release from target cells treated with 1% sodium dodecyl sulfate. The spontaneous release was 5–10% of the total cpm. NK activity was expressed as percent lysis with the standard deviation (SD).

Assay for Anti-viral Activity Levels of interferon (IFN) in the culture supernatants were measured in terms of their ability to inhibit cytopathic effects of VSV on mouse L-929 cells (kindly given by Prof. Y. Ito, Department of Microbiology, Mie University, School of Medicine). For IFN assay, a micro method was used in which a 96-well flat-bottomed plate was filled with 50 μl aliquots of a 1:2 dilution of the culture supernatants or the standard IFN in the first row of wells, and thereafter serial two-fold dilutions were made directly in the wells. The wells were then seeded with L-929 cells (5×10^4 cells/50 μl/well). Twenty-four hours later, the cells had reached confluence, and were challenged with VSV in 50 μl at a concentration of 10^2 50% tissue culture infectious doses ($TCID_{50}$) per ml, and the cytopathic effect was read microsopically after incubation for 30 hr. An IFN standard of known titer, equivalent to the NIH Reference IFN, was included on each plate to correct for fluctuations in the sensitivity of the system. Anti-viral units were expressed as international units (IU) per ml, calculated from the reciprocal of the highest dilution inhibiting 50% of the cytopathic effect.

Assay for Activity of Macrophage-activating Factor (MAF) Peritoneal exudate cells (PEC) from ICR mice injected intraperitoneally 4 days previously with 2 ml of sterile 10% proteose peptone (Difco) were washed out, centrifuged at 250*g* for 10 min at 4°, and resuspended in complete medium. Samples were taken for total and differential cell counts. Differential cell counts were made on Wright-stained (Diff-Quick; Kokusai-Shiyaku, Kobe) cell smears prepared by cytocentrifugation (Cytospin; Shandon Southern Instruments, Cheshire, UK). The PEC suspension containing 3×10^5 macrophages/0.1 ml was distributed into flat-bottomed 96-well culture plates and incubated for 2 hr at 37° in 5% CO_2 in moist air. Nonadherent PEC were then removed by repeated washing with complete medium. The adherent PEC were exposed to the culture supernatant in the presence of 10 ng/ml LPS, and incubated for a further 20 hr. These cells were washed intensively with complete medium. EL4 lymphoma cells maintained in *in vitro* culture

in this laboratory were labeled with ^{51}Cr, added to the culture wells (1×10^4 EL4 cells/well) containing the adherent PEC, and incubated for 20 hr at 37°. Cytotoxicity was estimated by measuring the ^{51}Cr release from target cells after 20 hr. The spontaneous release was less than 25% of the total ^{51}Cr on the target cells. The percentages of specific lysis were calculated by means of the same formula as described above for the NK assay. Activity of macrophage activating factor (MAF) was expressed as percent lysis with SD.

Results

Effects of MY-1 on the *in vitro* Cellular Responses Spleen cells were incubated for 20 hr with various concentrations of MY-1, or polyI:polyC taken as a control, and the NK activity of the cells and MAF activities of the culture supernatants were measured. As shown in Table I, all of the NK, MAF and IFN activities were elevated remarkably after the incubation with MY-1 in a dose-dependent manner, although the maximum levels of the activities were a little less than those after incubation with polyI:polyC.

Kinetics of NK Activation and MAF- and IFN-production by MY-1 Spleen cells were incubated with MY-1 for various times, and the NK activity of the cells and MAF and IFN activities of the culture supernatants were assayed. The results are shown in Table II. All of the NK, MAF and IFN activities reached the highest level after 6 hr of incubation. A significant elevation of NK activity was seen after 1 hr, while neither MAF nor IFN activity was observed within 3 hr.

Activities of the Nuclease Digests of MY-1 Activities of the RNase or DNase digest of

Table I. Augmentation of NK Activity and Induction of MAF and IFN with MY-1 and PolyI:polyC

Stimulant	Concentration (μg/ml)	NK activity (% lysis±SD)	MAF activity (% lysis±SD)	IFN activity (IU/ml)
Medium alone		4.0±0.8	3.8±1.3	<4
MY-1	1	10.2±0.4	11.8±2.8	16
	10	28.2±2.1	20.5±1.2	64
	100	45.4±3.7	26.9±5.4	128
PolyI:polyC	1	35.9±1.3	14.8±2.8	90
	10	56.5±1.2	23.4±0.9	181
	100	75.8±3.6	41.2±4.7	256

BALB/c mouse spleen cells (1×10^7/ml) were incubated with or without various concentrations of MY-1 or polyI:polyC at 37° for 20 hr. The culture was harvested, and the cells were tested for NK activity against RL♂1 cells. The supernatants were also assayed for MAF and IFN activities. SD (standard deviations) were calculated from triplicate experiments.

Table II. Time Courses of Activation of NK and Induction of MAF and IFN with MY-1 (100 μg/ml)

Time (hr)	NK activity (% lysis±SD)		MAF activity (% lysis±SD)		IFN titer (IU/ml)	
	Medium	MY-1	Medium	MY-1	Medium	MY-1
0	7.5±2.2	9.0±1.8	3.0±0.1	4.4±1.8	<4	<4
1	8.0±1.8	18.2±2.7	3.0±0.1	4.5±0.9	<4	<4
3	7.4±0.3	35.4±2.5	3.2±1.8	4.5±1.4	<4	<4
6	8.0±2.4	50.6±3.1	3.0±1.1	23.8±4.1	<4	90
20	9.4±0.9	55.5±2.2	3.5±2.1	25.6±2.0	<4	180

BALB/c mouse spleen cells (1×10^7/ml) were incubated with or without MY-1 (100 μg/ml) for various times. The culture was harvested, and the cells were tested for NK activity against RL♂1 cells. The supernatants were also assayed for MAF and IFN activities. SD (standard deviations) were calculated from triplicate experiments.

MY-1 were compared with those of MY-1. The results are shown in Table III. All of the NK, MAF and IFN activities of the RNase digest of MY-1 were almost the same as those of MY-1. No activity was seen in the DNase digest of MY-1.

Characterization of MAF and IFN Induced by MY-1 The culture supernatants of spleen cells incubated with MY-1 (100 μg/ml) for 20 hr were treated with anti-IFN-α/β or -γ antiserum, and their IFN and MAF activities were assayed. The results are shown in Table IV (Exp. 1). Treatment with anti-IFN-α/β, but not with anti-IFN-γ, diminished IFN activity. On the other hand, MAF activity was destroyed by treatment with anti-IFN-γ antiserum. The treatment with anti-IFN-α/β reduced MAF activity only slightly, although the unit of anti-IFN-α/β used was 100 times higher than that of anti-IFN-γ.

The culture supernatants of spleen cells incubated with MY-1 were dialyzed against

Table III. Comparison of Activities among MY-1, DNase Digest of MY-1 and RNase Digest of MY-1

Stimulant	Concentration (μg/ml)	NK activity (% lysis±SD)	MAF activity (% lysis±SD)	IFN titer (IU/ml)
Medium alone		4.6±0.8	1.2±2.9	<4
MY-1	1	11.0±1.8	13.4±2.0	16
	10	28.5±1.2	20.0±2.2	182
	100	37.1±3.4	24.9±2.4	256
RNase digest of MY-1	1	11.4±1.6	12.4±4.2	16
	10	31.9±3.3	18.2±3.3	182
	100	33.1±1.8	19.1±2.9	256
DNase digest of MY-1	1	4.1±1.9	0.3±0.8	<4
	10	3.4±1.3	3.8±3.9	<4
	100	4.4±0.8	2.2±1.6	<4

BALB/c mouse spleen cells (1×10^7/ml) were incubated for 20 hr with either MY-1, RNase digest of MY-1 or DNase digest of MY-1 at the concentrations indicated. The culture was harvested, and the cells were tested for NK activity against RL♂1 cells. The supernatants were also assayed for MAF and IFN activities. SD (standard deviations) were calculated from triplicate experiments.

Table IV. Effect of Anti-IFN-α/β or Anti-IFN-γ, and/or Acidic Treatment of IFN and MAF Activities

Exp.	Treatment	IFN titer (IU/ml)	MAF activity (% lysis±SD)
1	Medium	256	29.0±2.7
	Anti-IFN-α/β (1,000 U)	16	23.2±1.3
	Anti-IFN-γ (10 U)	282	1.5±0.3
2	Dialysis at pH 7	128	27.5±1.2
	Dialysis at pH 2	110	4.3±2.8
	Dialysis at pH 2 plus anti-IFN-α/β (1,000 U)	6	1.6±2.3
	Dialysis at pH 2 plus anti-IFN-γ (10 U)	103	0.8±2.9

Experiment 1: The culture supernatant from BALB/c mouse spleen cells incubated with MY-1 (100 μg/ml) for 20 hr was treated with either anti-IFN-α/β antiserum or anti-IFN-γ antiserum for 30 min, and then assayed for IFN and MAF activities. Experiment 2: A similar culture supernatant was dialyzed at either pH 7 or pH 2. After the dialysis at pH 2, the supernatant was treated with anti-IFN-α/β or anti-IFN-γ. All of these supernatants were then dialyzed against medium at pH 7 and tested for IFN and MAF activities. SD (standard deviations) were calculated from triplicate experiments.

buffers (pH 7 or 2), and then IFN and MAF activities were assayed. After the dialysis, some of the samples were also treated with anti-IFN antisera. The results are shown in Table IV (Exp. 2). MAF activity was destroyed by the treatment with pH 2 buffer, while IFN activity was not influenced. IFN activity of the material pretreated at pH 2 was destroyed by further treatment with anti-IFN-α/β, but not influenced by that with anti-IFN-γ.

The effects of the anti-IFN-γ antisera on MAF activity of the culture supernatant were studied more quantitatively. A culture supernatant of spleen cells (1×10^7 cells/ml) preincubated with MY-1 (100 μg/ml) for 20 hr was serially diluted, and each dilution was treated with either anti-IFN-α/β (1,000 U), anti-IFN-γ (10 U), monoclonal anti-IFN-γ (1 U) or medium alone. MAF activities of the treated samples are shown in Fig. 1. MAF produced from the spleen cells stimulated with MY-1 was neutralized with either the conventional or the monoclonal anti-IFN-γ antiserum but not with the anti-IFN-α/β antiserum.

Characterization of the Cells Responding to MY-1 To examine whether T lymphocytes are responsible for the activities of MY-1, spleen cells were incubated with either anti-Thy1.2, anti-Lyt1.2, anti-Lyt2.2 antiserum or medium alone at 4° for 40 min, then supplemented with rabbit complement, and incubated at 37° for a further 40 min. These cells were washed, and incubated with or

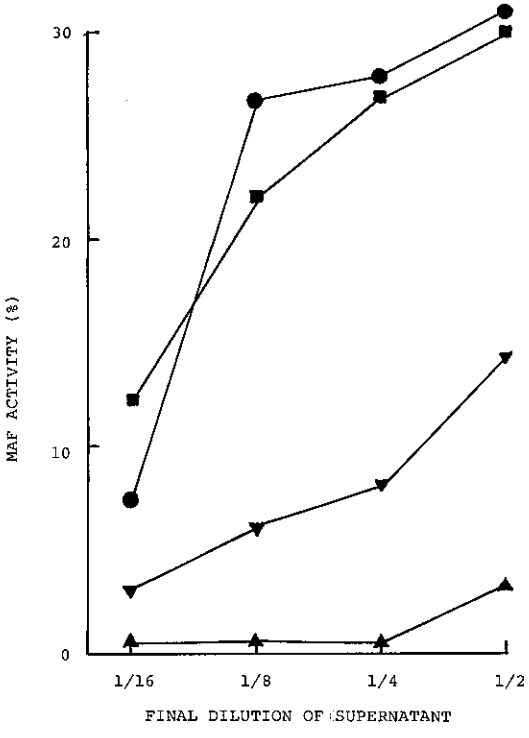

Fig. 1. Effect of anti-IFN-α/β or anti-IFN-γ on MAF activity of the culture supernatant. BALB/c mouse spleen cells (1×10^7/ml) were incubated with MY-1 (100 μg/ml) at 37° for 20 hr, and the supernatant was harvested. Then the supernatant was serially diluted, and each dilution was treated with either anti-IFN-α/β (1,000 U, ■), anti-IFN-γ (10 U, ▲), monoclonal anti-IFN-γ (1 U, ▼); or medium (●) for 1 hr. The treated supernatants were tested for MAF activity.

Table V. Effect of Pretreatment of Spleen Cells with Anti-asialo GM_1 Antiserum

Treated with	MY-1 (μg/ml)	NK activity (% lysis±SD)	MAF activity (% lysis±SD)	IFN titer (IU/ml)
Medium	0	5.3±0.7	2.4±0.6	4
	100	24.5±3.3	25.8±2.1	128
Anti-asialo GM_1+C	0	4.0±2.2	3.2±1.8	4
	100	4.4±2.2	22.8±3.5	9
NRS+C	0	3.8±1.3	2.8±1.3	4
	100	26.1±2.5	23.7±2.4	90

BALB/c mouse spleen cells (1×10^7/ml) were treated with either anti-asialo GM_1 antiserum, or normal rabbit serum (NRS), plus rabbit complement (C), or medium alone. The cells were washed, and then incubated with or without MY-1 for 20 hr. The cells were washed, and tested for NK activity. The supernatants were assayed for MAF and IFN activities.

Table VI. Effect of MY-1 on Nonadherent and Adherent Fractions of Spleen Cells

Spleen cell fraction	NK activity (% lysis±SD)		MAF activity (% lysis±SD)		IFN titer (IU/ml)	
	Medium	MY-1	Medium	MY-1	Medium	MY-1
Whole	3.5±2.1	27.5±4.3	4.2±0.8	23.8±3.2	4	180
Nonadherent	2.9±1.1	5.4±2.1	3.3±1.9	3.9±0.6	4	4
Adherent	2.1±0.8	7.5±3.3	3.6±2.1	18.8±3.1	4	8
Reconstituted	0.1±2.4	25.4±4.8	2.1±1.4	22.6±3.8	4	180

BALB/c mouse spleen cells were separated into nonadherent and adherent cells by the procedures described in the text, and each type was adjusted to a concentration of 1×10^7/ml. The whole cells were reconstituted by mixing equal volumes of the 2 fractions. Whole spleen cells, the fractionated cells and the reconstituted cells (1×10^7/ml) were each incubated with MY-1 (100 μg/ml) for 20 hr. The cells were washed and tested for NK activity. The culture supernatants were assayed for MAF and IFN activities.

without MY-1 at 37° for 20 hr. Although the data are not shown here, none of these treatments affected the MY-1 activities; NK, MAF and IFN activities were all augmented to similar levels relative to those of the untreated controls.

The responses of spleen cells from BALB/c mice and *nu/nu* and *nu/+* mice of BALB/c background to MY-1 were compared. All of the cells responded almost equally well to MY-1. Exceptionally, the NK activity of normal *nu/nu* spleen cells after incubation without MY-1 was a little higher than that of *nu/+* cells (6.5±4.2% and 3.2±1.3%, respectively), and the former responded more strongly to MY-1 than the latter (51.1±6.3% and 33.3±2.7%, respectively).

To characterize further the cells responsive to MY-1, spleen cells were pretreated with anti-asialo GM_1 antiserum plus complement and then incubated with MY-1. The results are shown in Table V. The cells pretreated with the antiserum plus complement showed no increase in either NK activity or IFN production after the stimulation with MY-1, but produced MAF as effectively as the control cells.

Adherent and nonadherent fractions of spleen cells were examined for response to MY-1. The nonadherent fraction contained less than 0.6% macrophages when measured by latex bead ingestion assay. As shown in Table VI, cells of this fraction showed no response to MY-1. When the adherent spleen cell fraction was incubated with MY-1, MAF was produced, but neither NK nor IFN activity was enhanced. When the adherent and nonadherent fractions were mixed, all of the responses to MY-1 were recovered.

Discussion

Our previous study indicated that administration of MY-1 *in vivo* augmented NK activity.[3] As shown here, this was also true *in vitro*; NK activity of normal spleen cells was augmented remarkably by incubation with MY-1. In addition, it was found that murine spleen cells stimulated with MY-1 *in vitro* produced factors which rendered macrophages cytotoxic and inhibited viral plaque formation.

MY-1 is a nearly pure fraction of nucleic acid (70.0% DNA and 28.0% RNA) with only traces of contamination with proteins, polysaccharides and lipids.[1] To determine the essential component responsible for MY-1 activities, activities of its RNase digest, composed of 97.0% RNA,[1] were compared with those of MY-1. As shown in Table III, DNA contained in MY-1 was responsible for its ability to activate NK cells and to induce factors possessing MAF and IFN activities. It is known that natural or synthetic double-stranded RNA stimulates the release of IFN and induces resistance to viral infection.[4-7] Herberman and his co-workers presented evidence that NK activity was markedly augmented by injection of a certain synthetic

RNA, polyI:polyC, but not by other single-stranded polyribonucleotides.[8,9] However, very little has been reported on the ability of DNA to induce IFN. More than 20 years ago, a few laboratories reported the antiviral action of DNA isolated from calf thymus or salmon testis,[10,11] but, afterwards, the validity of their nucleic acid hypothesis was questioned.[12] Since the physicochemical properties of the DNA contained in MY-1 have been well characterized,[1] this paper may be the first report that a soluble form of DNA can augment NK activity and induce factors with MAF and IFN activities.

Characterization of the factors released in the culture supernatant of the spleen cells incubated with MY-1 was carried out. As shown in Table IV and Fig. 1, MAF activity was destroyed almost completely by treatment with anti-IFN-γ antiserum or under acidic conditions (pH 2), but was not affected by anti-IFN-α/β antiserum. On the other hand, anti-viral activity was completely destroyed by anti-IFN-α/β, but not by anti-IFN-γ or by pH 2 treatment. These facts indicate that the MAF activity in the culture supernatant is attributed to IFN-γ and the anti-viral activity is due to IFN-α/β. It was estimated from a neutralization experiment with anti-IFN antiserum that spleen cells (1×10^7) stimulated with MY-1 (100 μg) for 20 hr produced more than 200 U or IFN-α/β. On the other hand, IFN-γ released into culture supernatants could not be detected by the viral plaque inhibition technique, but could by measuring MAF activity, which is a more sensitive assay method for IFN-γ.

Depletion of Thy1.2, Lyt1.2 or Lyt2.2 positive cells from spleen cells had no influence on the activities of MY-1 to augment NK activity and to produce MAF and IFN. Athymic nude mouse spleen cells also responded well to MY-1. These results indicate that mature T lymphocytes are not necessary for the response to MY-1.

When spleen cells were pretreated with anti-asialo GM_1 antiserum plus complement and then incubated with MY-1, neither NK activity nor IFN production was seen, while production of MAF (=IFN-γ) was not influenced (Table V). Interestingly, the nonadherent cell fraction never responded to MY-1, but the adherent cell fraction responded to it and produced MAF (Table VI). When both fractions were recombined, all of the responses were recovered. These results suggest that asialo GM_1-positive cells are precursors of NK, and both nonadherent and adherent cells are required for their activation by MY-1. The nonadherent cells may be responsible for the production of IFN-α/β, and the adherent cells may be responsible for the production of IFN-γ, although the adherent cells employed in this experiment contained a relatively large number of nonphagocytic cells as described in "Materials and Methods." Since many reports have shown that MAF/IFN-γ is induced in T cells or NK cells by stimulation with T cell mitogens or specific antigens,[13] further investigations are required to elucidate the target cells for MY-1.

Acknowledgments

This work was supported in part by Grants-in-Aid for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare, and funds from the Japan Health Sciences Foundation. The authors thank Ms. Mariko Takekawa for her technical assistance, and Ms. Noriko Katsu and Miss Kazumi Takahashi for their secretarial work.

(Received March 25, 1988/Accepted June 4, 1988)

References

1) Tokunaga, T., Yamamoto, H., Shimada, S., Abe, H., Fukuda, T., Fujisawa, Y., Furutani, Y., Yano, O., Kataoka, T., Sudo, T., Makiguchi, N. and Suganuma, T. Antitumor activity of deoxyribonucleic acid fraction from *Mycobaterium bovis* BCG. I. Isolation, physicochemical characterization, and antitumor activity. *J. Natl. Cancer, Inst.*, **72**, 955–962 (1984).

2) Shimada, S., Yano, O, Inoue, H., Kuramoto, E., Fukuda, T., Yamamoto, H., Kataoka, T. and Tokunaga, T. Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. II. Effects on various syngeneic mouse tumors. *J. Natl. Cancer Inst.*, **74**, 681–688 (1985).

3) Shimada, S., Yano, O. and Tokunaga, T. *In vivo* augmentation of natural killer cell activity with a deoxyribonucleic acid fraction of BCG. *Jpn. J. Cancer Res. (Gann)*, **77**, 808–816 (1986).

4) Lampson, G. P., Tytell, A. A., Field, A. K., Nemes, M. M. and Hilleman M. R. Inducers of interferon and host resistance. I. Double stranded RNA from extracts of *Penicillium funiculosum*. *Proc. Natl. Acad. Sci. USA*, **58**, 782–789 (1967).
5) Field, A. K., Tytell, A. A., Lampson, G. P. and Hilleman, M. R. Inducers of interferon and host resistance. II. Multi-stranded synthetic polynucleotide complexes. *Proc. Natl. Acad. Sci. USA*, **58**, 1004–1010 (1967).
6) Vilcek, J., Ng, M. H., Fredman-Kien, A. E. and Krawciw, T. Induction of interferon synthesis by synthetic double-stranded polynucleotides. *J. Virol.*, **2**, 648–650 (1968).
7) Colby, C. and Chamberlin, M. J. The specificity of interferon induction in chick embryo cells by helical RNA. *Proc. Natl. Acad. Sci. USA*, **63**, 160–167 (1969).
8) Oehler, J. R., Lindsay, L. R., Nunn, M. E., Holden, H. T. and Herberman, R. B. Natural cell-mediated cytotoxicity in rats. II. *In vivo* augmentation of NK cell activity. *Int. J. Cancer*, **21**, 210–220 (1978).
9) Oehler, J. R. and Herberman, R. B. Natural cell-mediated cytotoxicity in rats. III. Effects of immunopharmacologic treatments on natural reactivity and reactivity augmented by polyinosinic-polycytidylic acid. *Int. J. Cancer*, **21**, 221–229 (1978).
10) Rotem, Z., Cox, R. A. and Isaacs, A. Inhibition of virus multiplication by foreign nucleic acid. *Nature*, **197**, 564–566 (1963).
11) Jensen, K. E., Neal, A. L., Owens, R. E. and Warren, J. Interferon responses of chick embryo fibroblasts to nucleic acids and related compounds. *Nature*, **200**, 433–434 (1963).
12) Isaacs, A. Studies on interferon. *Aust. J. Biol. Med. Sci.*, **43**, 405–412 (1965).
13) Johnson, H. M. Mechanism of interferon production and assessment of immunoregulatory properties. *In* "Lymphokines," ed. E. Pick and M. Landy, Vol. 11, pp. 33–46 (1985). Academic Press, New York.